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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification 4 :</b> A61K 45/06, 31/00, 33/06 A61K 37/02 // A01N 1/02	<b>A1</b>	<b>(11) International Publication Number:</b> WO 86/ 00812 <b>(43) International Publication Date:</b> 13 February 1986 (13.02.86)
<b>(21) International Application Number:</b> PCT/SE85/00296 <b>(22) International Filing Date:</b> 30 July 1985 (30.07.85) <b>(31) Priority Application Number:</b> 8403912-2 <b>(32) Priority Date:</b> 30 July 1984 (30.07.84) <b>(33) Priority Country:</b> SE  <b>(71) Applicant (for all designated States except US):</b> PHARMACIA AB [SE/SE]; Rapskatan 7, S-754 50 Uppsala (SE). <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only) :</b> ARFORS, Karl-Erik [SE/SE]; Skogsmyrsvägen 5, S-752 45 Uppsala (SE). GERDIN, Bengt [SE/SE]; Björkhagsvägen 27 B, S-752 46 Uppsala (SE).		<b>(74) Agent:</b> FOGELBERG, Lennart; Stenhagen Patentbyrå AB, Karlavägen 18, S-114 31 Stockholm (SE).  <b>(81) Designated States:</b> AT (European patent), AU, BE (European patent), CH (European patent), DE (European patent), DK, FI, FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), NO, SE (European patent), US.  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> A DRUG KIT OR DRUG COMPOSITION FOR USE IN PREVENTING AND TREATING ISCHAEMIC CELL DAMAGE AND PREPARATION THEREOF  <b>(57) Abstract</b>  A drug kit or drug composition for use in preventing and treating ischaemic cell damage comprises: a) at least one plasma volume expander; b) at least one low molecular, physiologically acceptable hydroxyl radical scavenger; c) at least one physiologically acceptable and water-soluble magnesium salt; and d) at least one organic compound active as a calcium blocking agent dissolved in a carrier, either <i>per se</i> or in one or several combinations.		

A drug kit or drug composition for use in preventing and treating ischaemic cell damage and preparation thereof.

The present invention relates to a drug kit or drug composition for use in preventing and treating ischaemic cell damage.

5.

When circulation of the blood collapses and ischaemia occurs in peripheral body organs, particularly in the brain, a large number of pathophysiological changes take place. In present clinical practice it is only possible to treat measurable pathophysiological changes, for example changes in blood volume, impaired cardiac function, central acidosis, etc. In such cases each change has been treated individually and it can be said generally that present day therapy for the resuscitation of an organ is mainly directed towards re-establishing blood circulation.

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The present invention is based on the concept that incurable tissue damage can be caused as a result of unfavourable conditions created when re-establishing the blood circulation to a body organ.

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According to one aspect of this concept the transportation of calcium into and out of a cell is of great significance. The transportation of calcium into and out of a cell normally takes place while maintaining externally of the cell a calcium concentration which is 1000 times greater than the calcium concentration inside the cell. When a deficiency in energy occurs as a result of ischaemia, the calcium gradient cannot be maintained, and calcium will consequently leak into the cell. Calcium is taken up in the cell by the mitochondria, resulting in serious disturbances in energy production. When blood again starts to flow, calcium will enter the cell in still greater quantities, while transportation of calcium from the cell is impaired due to the fact that the build-up of energy in the cell is inhibited by the high calcium content thereof. This greatly increases the load on the mitochondria, which can lead to incurable cell damage and cell death.

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lower than 300,000 Daltons. It is well known in the art that the average molecular weight  $\bar{M}_w$  chosen depends on the high molecular substance used. Examples of such plasma expanders are plasma-albumin and substances based on dextran, starch derivatives or gelatine derivatives. The dextran products normally have an average molecular weight  $\bar{M}_w$  within the range of 30,000 to 80,000 Daltons. Examples of starch derivatives for this purpose include hydroxyethyl starch having an average molecular weight  $\bar{M}_w$  within the range of 40,000 - 400,000 Daltons, e.g. in the order of 200,000 Daltons. A number of different gelatine derivatives of varying average molecular weights  $\bar{M}_w$  are also used for this purpose. (A review of some plasma volume expanders is found, for example, in the book "Blood Replacement" by U.F. Gruber, Springer Verlag, Berlin-Heidelberg-New York 1969). Of these plasma volume expanders, those based on dextran are primarily preferred.

The concentration of plasma volume expander in the solution in which it is present is chosen so that subsequent to being optionally mixed with one or more solutions incorporated in the kit, the solution injected into the patient will have a plasma-volume-expander concentration which is normal in the use of the substance in question. The plasma volume expander solution of the invention usually has a concentration of 1-15 g/100 ml, such as 2-12 g/100 ml, for example 3-10 g/100 ml.

A common requirement of the hydroxyl radical scavengers which can be used in accordance with the invention is that they are physiologically acceptable and have a molecular weight beneath 10,000 Daltons, preferably beneath 1,000 Daltons. Hydroxyl radical scavengers which have a molecular weight above 10,000 Daltons as a rule have a poor effect. A suitable hydroxyl radical scavenger is soluble in water at physiological pH and ion strengths. It normally includes a functional structure selected from aromatic or aliphatic thiol (-SH), alcoholic and phenolic hydroxyl (-OH) and nitrogen-containing structures, such as primary amine (-NH<sub>2</sub>) secondary amine (-NH-) and imine (=NH). The hydroxyl radical scavenger

The organic compounds acting as calcium blockers are normally of low molecular weight, with a molecular weight beneath — 2000 Daltons. They are defined by their ability to prevent the migration of calcium ions into cells. Cf. "Calcium Blockers" (edited by Flaim, S.F. et al; Urban-and Scharzenberg. Baltimore-Munich, 1983). The compounds in question may be of highly different structure, nifedipine, nimodipine, verapamil, diltiazem, lidoflazine, flunarazine and analogous compounds can be mentioned by way of example. The calcium blockers used in accordance with the invention may be soluble in water and/or in fat. Verapamil(5-[(3,4-dimethoxyphenylethyl)methylamino]-2-(3,4-dimethoxyphenyl)-2-isopropylvaleronitrile) is an example of a water-soluble calcium blocker, while an example of a fat-soluble calcium blocker is lidoflazine(4-[4,4-bis(4-fluorophenyl)butyl]-N-(2,6-dimethylphenyl)-1-piperazine acetamide). When a fat soluble calcium blocker is used in accordance with the invention, it is advantageously included in the kit as a component separate from the plasma volume expander. According to one aspect of the invention, this enables lidoflazine to be administered in a separate injection when using the drug kit. In this variant of the invention, the fat-soluble calcium blocker may be dissolved in, for example,:

I. A mixture of water and ethanol in an amount which is physiologically acceptable for the purpose. When the calcium blocker has the nature of an amine, the mixture can be acidified, to increase solubility. It is essential in this respect that acidification of the mixture is adapted to the pH and buffer capacity of the remaining kit components to be used on the occasion of the treatment. The mixture is advantageously acidified with acetic acid, hydrochloric acid, or some other physiologically acceptable acid. The mixture may also contain glycerol.

II. Physiologically acceptable fat emulsions used for parenteral nutrition (a number of such emulsions are described, inter alia, in patent literature; cf. for example the U.S.

in the range of 7.0 - 10.0, preferably 7.4 - 9.2.

The drug kit or composition according to the invention preferably also includes a diuretic agent, particularly an osmotic diuretic agent, and/or an anti-oedema substance. Since in addition to being an hydroxyl radical scavenger, mannitol is also able to fulfil the function of both a diuretic and an anti-oedema substance, mannitol is a preferred substance in the present context. Sorbitol or glycerol can be used as a diuretic agent, either instead of or together with mannitol. The quantities in which a diuretic agent and anti-oedema substance is used is dependent on the substance utilized, and may thus vary within wide limits. In the case of an osmotic diuretic agent, the quantities used may lie within the range 5-150 g, otherwise 0.1-200 mg. In the case of the anti-oedema substance a corresponding range may be 5-150 g.

It may also be of advantage to incorporate in the kit or the composition according to the invention an xanthine oxidase inhibitor, such as allopurinol for example, (50 mg - 5 g, depending on which is chosen), and/or a superoxide radical scavenger, such as superoxide dismutase for example, and/or an hydrogen peroxide inactivator, such as catalase for example, and/or a substance which binds iron in a solid complex, such as desferrioxamine or diethylenetriamine-pentaacetic acid or ethylenediamine-di(o-hydroxyphenylacetic acid), or a phytic acid derivative.

The quantities quoted above in respect of the diuretic agent, anti-oedema substance and xanthine-oxidase inhibitor apply to each occasion of treatment.

The active components included in the drug kit or drug composition are present in the form of a single solution or a plurality of solutions. Precisely how they are combined is determined, inter alia, on the grounds of solubility and stability, even though for practical reasons the aim is to place

syringe.

The concentration in which the active components are present are selected so as to maintain the mutual proportions between the aforementioned quantities. In the aforesaid preferred embodiments, the concentration of hydroxyl radical scavenger and magnesium salt corresponds to the aforementioned quantities per 500 ml of solution. The same applies to the calcium blocker, when it is incorporated in the same solution as these two substances. When it is present in a separate solution (C), the calcium blocker concentration may be from 10 to 100 times greater than in the previous case, due among other things to the solubility conditions.

When calculated on the basis of a patient weighing 70 kg, the kit components are normally administered to the patient in a total solution volume of 500-600 ml.

When using a drug kit according to the invention in which the calcium blocker is included as a separate unit (C), this unit is the first to be injected into the patient. It is desirable that this injection can be given relatively quickly. In those cases where metabolic acidose prevails, as with a cardiac arrest for example, solution (B) is used to correct the pH of the patient. The solution (B) may be mixed with the solution (A) immediately or shortly before being used. The mixture, or the solutions (A) and (B) each per se, is or are then administered to the patient as soon as possible after having injected the patient with (C). In the absence of metabolic acidose, only solution (A) is administered.

When using a drug composition according to the invention in which a plasma volume expander, an hydroxyl radical scavenger, magnesium salt and a calcium blocker are present in a common solution separate from a buffer solution (B), this common solution is injected into the patient separately or in mixture with (B). The solution (B) is only used in the case of metabolic acidose.

Solution B

There were used for this solution 50 ml of a conventional commercial buffer solution having a pH of 9.2 and containing 20 g trometamol with a buffer capacity of 150 mmol (Addex<sup>®</sup> THAM form Pharmacia Infusion AB, Uppsala, Sweden).

Solution C

80 mg lidoflazine were dissolved in 1.0 g ethanol (99.5%), 0.1 g concentrated acetic acid and 1.5 g glycerol, and was diluted up to 10 ml with distilled water. The solution was sterilized by sterile filtration and poured into a 10 ml ampoule under aseptic conditions.

The solutions A, B and C were then packed in a box, as a unit.

EXAMPLE 2Pharmacological tests

The tests were carried out with a rat model, which gives an incomplete cerebral ischaemia with a cortical flow < 5% of the normal flow, and a flow in the brain stem which is about 30% of the normal flow. This is effected by squeezing the two carotid arteries while simultaneously lowering the blood pressure to 50 mm Hg, by bleeding. The method has been described by Nordström C.H. and Siesjö B.K., Stroke 9, 327-335 (1978).

Wistar-rats weighing 300-400 g and fasted overnight were used in the tests. The rats were anaesthetized with 4% Fluothane<sup>®</sup> (ICI-Pharma AB, Gothenburg, Sweden), 30% O<sub>2</sub>/70% N<sub>2</sub>O, intubated and connected to a respirator. The vena jugularis externa was uncovered. Celocurine (5 mg/kg) was injected and a catheter was placed in vena cava superior. Catheters were also placed in the tail artery and in a tail vein for measuring blood pressure and infusion, respectively. EEG-electrodes were applied and finally 5 ml 0.9% NaCl were administered intraperitoneally and 100 IU heparin intravenously. The supply of Fluothane<sup>®</sup> was cut-off, whereafter blood gases, pH and the



stable breathing was observed.

Of 10 test animals in each group, the mortality of the control group was 60%. The corresponding figure in the group treated with a drug kit according to the invention was 20%. No significant differences were observed with regard to average arterial blood pressure, blood gas or blood sugar. With regard to the pH of the blood, it was observed that the blood-pH of the animals in the group treated with a drug kit according to the invention fell after the ischaemic period to a lesser extent than that of the animals in the control group, this being attributed to the buffer capacity of the drug kit according to the invention.

8. A drug kit or composition according to Claim 6, characterized in that the anti-oedema substance is mannitol.
9. A drug kit or composition according to any one of Claims 1-8, characterized in that it also includes an xanthine oxidase inhibitor and/or a superoxide radical scavenger and/or a hydrogen peroxide inactivator and/or an iron-binding substance.
10. A drug kit or composition according to any one of Claims 1-9, characterized in that it also includes a physiologically acceptable buffer system.
11. Process for the preparation of a drug kit or drug composition for use in preventing and treating ischaemic cell damage, characterized by dissolving
  - a) at least one plasma volume expander;
  - b) at least one low molecular, physiologically acceptable hydroxal radical scavenger;
  - c) at least one physiologically acceptable and water-soluble magnesium salt; and
  - d) at least one organic compound active as a calcium blocking agentin a carrier, either per se or in one or several combinations.

## FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

II

Fields searched (cont).US Cl 424:154, 180, 250V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE <sup>1</sup>

This International search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers..... because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claim numbers..... because they relate to parts of the International application that do not comply with the prescribed requirements to such an extent that no meaningful International search can be carried out, specifically:
3. ☐ Claim numbers..... because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(e).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING <sup>2</sup>

This International Searching Authority found multiple inventions in this International application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International search report covers all searchable claims of the International application.
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this International search report covers only those claims of the International application for which fees were paid, specifically claims:
3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

## Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.